To determine whether mycophenolic acid Area under the Curve Correlates with disease activity in pediatric lupus nephritis patients treated With Mycophenolate Mofetil

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE RULES AND REGULATIONS FOR THE MD BRANCH II (PEDIATRICS) DEGREE EXAMINATION OF THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY TOBE HELD IN APRIL2013

CERTIFICATE

This is to certify that the dissertation entitled "To determine whether mycophenolic acid area under the curve correlates with disease activity in pediatric lupus patients treated with mycophenolate mofetil" is the original work of Dr. Kirubakaran Navamani towards the M.D branch II (Pediatrics) degree examination of The Tamil Nadu Dr. M.G.R Medical University, Chennai to be held in April 2013.

Signatures

Guide:	**Co-Guide:**	**Co-Guide**:
Dr. T. Sathish Kumar MD, DCH	Dr. Indira Agarwal MD, FISN	Dr.Ratna Prabha MD
Professor	Professor & Head	Asst Professor
Dept of Pediatrics –II	Dept of Pediatrics-II	Dept of clinical Pharmacology
Christian Medical College	Christian Medical College	Christian Medical College
Vellore-632004	Vellore- 632004	Vellore-632004

Head of the department

Dr. Anna Simon MD, DCH

Professor and head

Department of Paediatrics

Christian Medical College

Vellore-632004

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INTRODUCTION

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disorder with multisystem involvement. It predominantly involves adolescent girls. 20% of all SLE begin in childhood after 5 years of life. It is more predominant in females with a female to male ratio of 8:1. The ratio lowers down to 2:1 in the pediatric age group(1,2).

The Etiology of systemic lupus erythematosus is unknown. Environmental factors (UV light, drugs, toxins and infection) may play a role in the genetically predisposed population. The pathogenesis is complex. The autoantibodies, polyclonal B cell activation and the T cell dysfunction contributes significantly to the disease activity (3). Lupus nephritis is very common among the pediatric population. It is reported that about 40-75% of patients diagnosed to have SLE, develop lupus nephritis within 5 years of diagnosis and almost all patients have a degree of glomerular abnormality. The risk of development of end stage renal disease is 18-50%(44,5). High dose corticosteroids have improved the course of lupus nephritis. However in the last 20 years additional treatment including the use of cytotoxic therapy has increased the 10 year survival to 80% (6).

Mycophenolate Mofetil (MMF) is an immunosuppressant that is routinely given for treatment of acute graft rejection and for prophylaxis following solid organ transplant. MMF is the norphlinoethyl ester of mycophenolic acid (MPA). MPA inhibits the denovo purine synthesis.

Lymphocytes are selectively inhibited because lymphocytes rely on de novo synthesis of purines (7). MMF also depletes the lymphocytes and monocytes of guanosine triphosphate by selectively inhibiting inosine monophosphate dehydrogenase(8). Successful use of MMF in lupus nephritis has been described in various studies(7,9). MMF is also known to produce lower rates of infection and cytopenia compared to patient who are treated with cyclophosphamide(10). However the optimum dose of MMF in lupus nephritis has not yet been defined. The pediatric post organ dose of 30mg/kg twice daily or 600mg/m2 twice daily is being followed for the treatment of lupus nephritis(11). A fixed dose of 0.5 g to 1.5 g bid adjusted to the tolerance of the individual is also being followed in the treatment of lupus nephritis(12). A target concentration of MPA that is aimed at is 30-60 mg h/L. Also there is an inter-individual variability of MPA concentrations for a given dose of MMF and the MPA concentrations also depend on whether the drug is given along with other drugs like cyclosporine or tacrolimus(13). We try to demonstrate the inter-individual variability of MPA concentrations for a given dose of drugs and also co-relate the association between the disease activity as measured by the SLE disease activity index (SLEDAI) and the concentration of the drug.

LITERATURE REVIEW

LITERATURE REVIEW:

HISTORY:

The history of lupus dates back to the 13th century when the physician Rogerius described the lesions as wolf bite. Lupus in Latin means wolf. Incidentally that is when the term lupus was coined(14). It was then considered to be a cutaneous disease. Only in the latter half of the 19th century was it recognized as a multisystem disease after the works of Kaposi.

DISEASE BURDEN:

Systemic lupus erythematosus is an autoimmune disorder with multisystem involvement and is potentially fatal. The prevalence of lupus ranges from 40 per 100,000 persons among the Europeans. Among blacks the prevalence is as much as 200 per 10,000 persons(15). In a study from Asia 15 to 20% of all lupus is diagnosed in the pediatric age group (16 years and less).The prevalence of lupus in the pediatric age group of the Asian population was found to be 6.3 to 10.3 per 100,000 (16).

ETIOLOGY:

The etiology of lupus is unclear. Around 90 % of lupus is females. Hence the contributory role of female sex hormones and the protective role of male sex hormones are possible. Buyon et al concluded that, the post menopausal women with SLE who received hormonal replacement with conjugated estrogen and progesterone were more prone for disease flare than the patients receiving placebo(17). But clinical trials which involves administration of dihydroepiandrosterone for the treatment of lupus has not been promising (18).

Genetic Factors

There is a possible genetic role in the etiology of SLE. It tends to occur in families. However there is no clear cut Mendelian inheritance. There is more occurrence of the disease in families. There is 2% increased risk of disease in a sibling with SLE. However in even monozygotic twins there is only 25% increased risk of disease and in dizygotic twins the risk is even lower(19). These rates mean that there is not enough evidence for a genetic role. Many genes have been identified after genome-wide genetic association studies in families with multiple lupus patients which possibly contribute to the disease(20). Certain genes for the major histocompatibility proteins namely HLA A1, B8 and DR3 are associated with lupus (21).

Early complement component(C1q, C2 or C4) deficiency is strongly associated with lupus (22). Family studies have identified many genes in patients with lupus. Their function is to code for components of the Immune system. A Scandinavian study showed that a single nucleotide polymorphism in two interferon-related genes coding for tyrosine kinase 2 and interferon regulatory factor 5(23). In mice studies Wakeland identified three genetic loci that are associated with lupus namely sle1, sle2 and sle3. Sle1 gene mediates the loss of immunological tolerance to nucleolar autoantigens. Sle 2 and Sle3 mediates B cell hyperactivity and T cell dysregulation respectively(20).

Environmental factors

Drugs like hydralazine, procainamide and quinidine are known to cause drug-induced lupus. The drug induced lupus presents with rash and joint pain and generally do not cause nephritis and CNS manifestation (24). Ultraviolet radiations has been identified as the most important environmental factor in causing lupus(25,26). Preceding viral infections are noted before the presentation of prior to a flare. Particular causative virus has not been described yet. However Ebstein-Barr(EBV) virus is possibly associated as disease presentation and the infection occurring simultaneously has been reported(27). A case control study showed that EBV DNA was present in 100% of cases with lupus.

Auto-antibodies in lupus

Autoantibodies play a major role in the pathogenesis of lupus. Kidneys of patients with lupus nephritis were demonstrated to have antibodies which bound to native double stranded DNA(Ds-DNA) (28). Anti-Ds-DNA is present in as many as 70% of patients with lupus. It is also present in about 0.5% of healthy individuals and in individuals with other connective tissue like rheumatoid arthritis(29). It is strongly associated with the disease activity(30).Patients with anti-ds-DNA positivity and clinically asymptomatic have a 80% chance of having active disease in 5 years(31). In other post mortem renal biopsy studies by Mannik et al there was evidence of auto antibodies against non DNA proteins like Ro (ribonucleoprotein complex), La (RNA binding protein), Sm (nuclear particles) and C1q(a subunit of C1) (32). Anti-ribosomal P antibodies are more common in childhood nephritis and is positive in severe nephritis(33). The presence of anti-RO or anti-LA or both during pregnancy is associated with 1 to 2 % risk of fetal heart block(34) .

Anti-N-methyl d-aspartate (NMDA) may be important in CNS lupus. Kawal showed that antibodies against NMDA and DNA produced cognitive impairment in patients with lupus and hippocampal damage in mice models (35). Anti Ro and anti-nucleosome antibodies are seen in cutaneous lupus. Anti-nucleosome antibody is seen in skin biopsy specimens in patients with no

active skin lesion but active nephritis(36). Hemolytic anemia and thrombocytopenia in lupus occurs secondary to antibody mediated destruction of platelets and red blood cells.(37)

There are two theories of damage caused by autoantibodies. Berden et al suggest that auto antibodies against the double stranded DNA (anti ds-DNA) bind to the nucleosome which are released into the cytoplasm and this compound deposits in the glomerular basement membrane thereby causing glomerulonephritis(38). In animal models this nucleosome- autoantibody complex is studies to initiate complement activation(39). The second theory states that both anti-nucleosome antibody and anti-ds-DNA both cross react with proteins in the kidney and thereby causing damage(40).

Clinical manifestations:

Systemic lupus erythematosus presents in children acutely and usually with multisystem involvement. Fever, musculoskeletal symptoms, fatigue and anorexia are the common presentation.

Arthritis is usually found in 50-75% of the individuals. The arthritis in lupus is characteristically non deforming and non erosive (41). Arthritis may be symmetrical, painful polyarthritis which affects both small and large joints. The ultrasound study in the joints affected showed tenosynovitis with thinning of the tendon(42). 20-30% of children have myalgia. Although myositis is rare when present makes it difficult to differentiate it from dermatomyositis.

Mucocutaneous involvement is seen in 60-80% of patients with pediatric SLE at the time of presentation. The rash associated with SLE is the malar rash a maculopapular rash which involves the malar area and the bridge of the nose. It is usually photosensitive and resolves without any residual scarring. A discoid lesion is uncommonly seen in lupus. It is photosensitive and occurs in the forehead and scalp (43). The malar rash that is known to be commonly

associated with the disease is not very frequently seen in Indian children. The presence is also not pathognomonic of the disease(44).The oral mucosal lesions are hyperemia and ulcerations usually seen in the hard palate and are characteristically painless. Mild alopecia may be the presenting feature. It can also be severe (45).

Hematological manifestations are seen in about 100% of patients. The ethnic background plays a large role in the incidence of hematological manifestation. Anemia is seen in 75% of the Indian children. Anemia of chronic disease is most commonly seen in SLE. The anemia is initially normocytic normochromic and later microcytic hypochromic. 30-40% has Coomb positivity. However only10-15% of them have overt hemolysis (46). 15 to 45% of patients have thrombocytopenia. It can be the presenting feature in 15% of the patients. Children who present with autoimmune thrombocytopenia should be evaluated routinely for lupus in view the high incidence(47). Leucopenia (both lymphopenia and granulocytopenia) is found in 20-40% of individuals(48). Coagulation abnormalities are seen. 20% of patients with pediatric SLE have lupus anticoagulant positivity. They usually have thromboembolic events. They have 20-30 times increased chance of have a thromboembolic event (49). The commonest cause for secondary antiphospholipid syndrome is systemic lupus erythematosus in children. Lupus may also manifest many years after presentation of primary antiphospholipid syndrome(50). It present as thromboembolism and venous events are more common than arterial events(49).

Among the cardiovascular manifestations Pericarditis with pericardial effusion is the most common. Mild pericardial effusion which is picked up by echocardiography is seen in1/3rd of the patients. However overt pericardial effusion is seen in 25-40% of the individuals(41,44).

Pleuropumonary involvement is seen in 25 – 75% of patients (51).Pleuritis is the most common manifestation. Acute lupus pneumonitis may present similar to infective pneumonia or pulmonary hypertension(52).5-10% of lung manifestations re pulmonary hemorrhages (53). Pulmonary hypertension secondary to pulmonary vascular disease is a rare but potentially fatal complication(54)

Involvement of the central nervous system is reported in a wide range of patients. It may occur in as low as 20% to about 90% of patients(55). The reason for this wide variation in the incidence of central nervous system complications is because of the discrepancy in the definition of certain central nervous system symptoms. The commonest neuropsychiatric manifestation is Lupus headache. Lupus headache is defined as an unremitting headache which requires narcotic treatment (56). 30-50% of neuropsychiatric manifestations are contributed by psychosis, They more commonly have visual hallucinations and less commonly auditory and tactile hallucinations in that order. They characteristically have preserved insight(57). 12 to 30% of patients with neuropsychiatric manifestations have cerebrovascular disease(58). 10-20% of children with CNS lupus have cerebral venous thrombosis and are almost universally associated with lupus anti coagulant(49).

Abdominal pain and diarrhea are the most common gastro intestinal manifestations(59). When associated with vasculitis if the gastrointestinal system the patients are at risk of developing an intestinal perforation(60). Pancreatitis is a rare complication presenting in about <5% of the cases. Patients usually present with vomiting and diffuse abdominal pain(61).

Ocular finding that is most associated with lupus is cytoid bodies. This is secondary to retinal vasculitis(62).

Infectious complications are extremely common and are a major cause of death. They create diagnostic and therapeutic challenges. In the presence of fever along with respiratory symptoms or central nervous system symptoms like seizures, altered behavior infection needs to be ruled out. If investigations show high counts with neutrophilic predominance which is unlike SLE sepsis needs to be ruled out. However counts may not be necessarily high in the setting of infection as patients with SLE may have apparently normal counts during infection as their baseline counts may be low. An elevated CRP also suggests infection. Opportunistic infections like tuberculosis and fungal also needs to be considered.

Lupus nephritis is very common among individuals with SLE. Almost all patients are reported to have some degree of glomerular abnormality. About 40-75% of patients with SLE develop clinical nephritis within 5 years of diagnosis(63). The risk of progression to end stage renal disease is 18-50% (4,5). The presentation of lupus nephritis can vary extensively from individual to individual. It can range anything between mild nephrologic abnormality to rapidly progressing and nephrotic syndrome. Hematuria and proteinuria are the most common. Hematuria is present in 67-100% of individuals with lupus nephritis whereas nephritic syndrome is present in about 50% at diagnosis. Hypertension and renal insufficiency is seen in 50% of affected individuals(5). Age related disease manifestations were studied. Children were found to have higher incidence of hypertension, proteinuria, hematuria, cellular casts and Creatinine(64).

Patients with hematuria and/or proteinuria may have any class of glomerulonephritis. The prognosis depends on the stage of renal involvement and it is of utmost importance in deciding the treatment (65). Children with silent disease may have major histopathological abnormalities. Hence the clinical picture always does not correlate with the renal histopathology(6). WHO developed a classification for lupus nephritis in 1973 which helps in prognosis and deciding on further therapy. The classification uses light microscopy, immunofluorescence and electron microscopy(6).

WHO has classified lupus nephritis as follows (66)

Class I : Normal glomeruli a) Normal by light microscopy b) immunological deposits by electron microscopy

Class II: Mesangiopathy. a) Pure mesangial widening with mild hypercellularity b) moderate hypercellularity

Class III: Focal and segmental glomerulonephritis a) active and necrotic lesions b) active and sclerosing lesion c) sclerosing lesions

Class IV: Diffuse proliferating glomerulonephritis (severe mesangial, mesagiocapillary or endocapillary and /or extensive subendothelial deposits) without segmental lesions b) with "active" necrotizing lesions c) with active and sclerosing lesions d) with sclerosing lesions

Class V: Diffuse membranous glomerulonephritis a) pure membranous glomerulonephritis b) associated with lesions of category IIa or IIb. C) Associated with lesions of category III (a-c) d) associated with lesions of category IV (a-d)

Class VI: Chronic sclerosing glomerulopathy

Treatment of lupus nephritis:

Clinical trials in the pursuit of treatment for SLE started as early as 1894 when Payne reported the beneficial effects of quinine in SLE. Brief period later salicylates were proved beneficial. But the break-through in the treatment of lupus was not attained until the 1950s when Hench reported the efficacy of cortisone in the treatment of rheumatological conditions like SLE (67) and ever since steroids have been the primary therapy in the treatment of SLE.

However the ideal treatment for SLE nephritis is unclear despite years of research primarily because of the basic pathophysiology. It is not clear whether the excess B cell activity or defective T cell suppressor activity or excess helper T cell activity is being dealt with(68).

With the advent of the recent treatment options the outcome of pediatric SLE has improved dramatically over the past years. The 10 year survival rate in the 60's was as low as 30% and in the early 90's it has improved to excess of 90%(69). Non renal causes like infections has replaced renal failure the common cause of death in Lupus(70). The treatment needs to be balanced between an aggressive initial therapy to control disease activity and a maintenance

therapy aimed at minimal aide effects. Delayed onset of treatment is associated with poorer outcome(71).

Therapy of class I, II nephritis:

Patients with class I nephritis are very rare and no specific treatment has been described. There is no specific treatment described for the treatment of class II nephritis either. Corticosteroid, as a long term treatment of lupus nephritis is not indicated. It is rather aimed at associated extra-renal manifestations of lupus(64). However there is need for long term follow up for the progression of disease

Therapy of class III, IV nephritis:

The course of the disease in class III lupus nephritis is the same as that of class IV when more than 40% of glomeruli are involved and hence the same aggressive therapy is needed(6). However if less than 20% of glomeruli are involved , the prognosis is quite good with <5% of patients progressing to end stage renal disease at the end of 5 years(72).

Patients with Class IV lupus nephritis (Diffuse proliferative lupus nephritis) is prone for hypertension , nephrotic syndrome and end stage renal failure. Prompt treatment is warranted in class IV lupus nephritis. Corticosteroids have dramatically changed the course of the disease. Recent studies have shown that low dose corticosteroids are as equal in efficacy as high dose corticosteroids. In fact high dose corticosteroids are associated with serious side effects (73).

Sterinberg et al assessed 111 children and concluded that the patients in the study arm which involved treatment with a cytotoxic drug plus low dose prednisolone had significantly better preservation of renal function compared to those treated only with high dose steroids(74).

Unlike adults treatment with corticosteroids and immunosuppressants are required in the treatment of Child hood SLE(75). Treatments that are currently used are antimalarials, corticosteroids, cyclophosphamide, azathioprine and mycophenolate mofetil.

Corticosteroids:

For over two decades the teaching was to treat diffuse proliferative glomerulonephritis with high dose corticosteroids. Recent studies show that high dose steroids are no better than low dose steroids and are associated with unwarranted side effects. In his randomized control trial Steinberg demonstrated that patients randomized to the groups treated with cytotoxic therapy in addition to steroids had better preservation of renal function that did the group treated with prednisolone alone(74). Pulse methylprednisolone administered intravenously as pulse doses leads to dramatic improvement in patients with acute deterioration of renal function(76). However the long term effect of this regimen in preserving renal function matched only prednisolone. Also intravenous methylprednisolone is associated with side effects like cardiac arrhythmias and cardiac arrest. Other side effects like flushing sensation, acute hypertension and acute psychosis are also associated(77).

Cyclophosphamide:

There is much evidence that combination of cyclophosphamide with steroids has better results in preserving the renal function than steroids alone[55]. Cyclophosphamide is metabolized in the liver to its active metabolite. The active metabolite alkylates the macromolecules(78). Initially oral regimens in the dose of 1-3mg/kg/day for 8-12 weeks were described. Currently monthly boluses at a starting dose of 750mg/m^2 is suggested to be less toxic than oral daily doses at 2mg/kg(79). The dose of cyclophosphamide may be increased to 100mg/m^2 if the WBC count remains more than 3000/mm^3. The duration of therapy after the initial control of disease is not well described. In his study Lehman used cyclophosphamide for 3 years and reported improvement in hemoglobin C3, C4 and Creatinine clearance(80)

Cyclophosphamide therapy is associated with the risk of toxicity which includes alopecia, bone marrow suppression , gonadal failure, hemorrhagic cystitis and development of malignancy(81).

Azathioprine:

Azathioprine is an antimetabolite that interferes with protein synthesis(78). It has been proved to be safe when given in the log-term. It is given in the doses of 2-2.5mg/kg per 24 hours. It may be used with prednisolone in the initial treatment of lupus nephritis(82) and later substituted for iv cyclophosphamide after 6 months if the disease is well controlled. It can also be given after completing 8-12 weeks of oral cyclophosphamide(64). When Azathioprine is given in combination with steroids the steroid dose has to be changed in the event of withdrawing Azathioprine as the disease may relapse. This is because of the steroid sparing effect of azathioprine(83) . Azathioprine is relatively safe, however long term administration causes bone marrow suppression which is reversible on discontinuation(84)

Cyclosporine A

It is a relatively new drug which is used in the event of steroid resistance or in severe steroid toxicity. It acts by interfering with the production of lymphokines produced by the lymphocytes(85). Cytotoxic T call recruitment is stopped by inhibiting production of interleukin-2 and thereby decreasing inflammation(64). Cyclosporin used along with steroids is known to decrease proteinuria and improve renal function with better growth rate as compared to patients treated with prednisolone and cyclophosphamide combination and its use alone(85). Side effects are minimal. Hypertension, transient elevation of serum Creatinine, hypertrichosis and gingival hyperplasia.(64)

Mycophenolate Mofetil:

Mycophenolate mofetil is a relatively new drug which has been used routinely in the prophylaxis against graft rejection and in its treatment in the post renal transplant patients. Mycophenolate mofetil (MMF) is immunosuppressive drug which acts by irreversibly inhibiting the enzyme inosine monophosphate dehydrogenase (IMPDH) and hence selectively inhibiting proliferation of T- cells and B cells as they require de-novo synthesis of purines(86). Mycophenolic acid (MPA) is the active form of the inactive prodrug mycophenolate mofetil (MMF). MMF is converted to MPA by liver, plasma and intestinal esterases (87). There are many recent studies which show that mycophenolate mofetil is as efficacious or atleast comparable with cyclophosphamide in the induction treatment of childhood systemic lupus erythematosus(88,89). MMF is also known to produce lower rates of infection and cytopenia compared to patient who are treated with cyclophosphamide(10).

The dose recommended in clinical practice is based on clinical trials for renal transplantation. It is a fixed dose of 2 to 3 grams per day in divided doses. Other evidences based on pharmacokinetic studies in children with autoimmune diseases, a dose of 900mg/m2 is suggested

in children. The dosage is lower than in patients who have undergone solid organ transplantation (1200 to 2400 mg/m2) who simultaneously receive calcineurin inhibitors. In renal transplantation therapeutic drug monitoring has been developed for individualization of doses. MPA area under the plasma concentration time curve from 0-12(MPA AUC_{0-12} hrs) has been successfully correlated in the outcome of patients who have undergone solid organ transplantation(90).

The MPA pharmacokinetics in patients with systemic lupus erythematosus is different from the patients who have undergone solid organ transplants who are on Cyclosporin A (91,92) . Patients with SLE often have a third peak in the AUC due to the absence of calcineurin inhibitors. Plasma concentration of MPA are reduced by cyclosporine as it inhibits the enterohepatic circulation of MPA(93,94). Target concentration of AUC over 12h of 30-60 mg h/l when measured with high performance liquid chromatography or 35 to 70 mg h/l when measured by enzyme multiplied immunotechnique is advised for patient undergoing renal transplant (95). (12).

A target concentration of MPA that is aimed at for the therapy of lupus nephritis is also 30-60 mg h/L. Also there is an inter-individual variability of MPA concentrations for a given dose of MMF and the MPA concentrations also depend on whether the drug is given along with other drugs like cyclosporine or tacrolimus(13).

The target concentrations of MPA in children treated with MMF for SLE is very important as this reflects the clinical outcome and the association with side effects. There are very few studies which have reported the inter-individual variability of MPA concentration and even fewer in children. Filler et al studied 5 children with autoimmune diseases and compared the pharmacokinetics of mycophenolate sodium (EC-MPS) with pharmacokinetics of mycophenolate mofetil (MMF). Factors affecting inter-individual variability are studied in transplanted individuals. Patients with renal insufficiency (creatinine clearance of <25ml/min) and low albumin (32g/l) have low MPA exposure because MPA clearance depends on its non protein bound fraction(96). Both hypoalbuminemia and renal insufficiency results in a high free fraction of MPA and hence increased clearance of MPA(92,97).To our knowledge the target concentration of MPA in children with Lupus nephritis has not been discussed till date.

AIMS

AIMS:

1) To determine whether MPA AUC $_{0\text{-}12}$ correlates with disease activity in children with lupus nephritis on mycophenolate mofetil

2) To demonstrate the inter individual variability of the MPA concentration in children taking MMF

3) To determine the independent variables that correlate with MPA AUC $_{0\text{-}12.}$

MATERIALS AND METHODS

Materials and Methods:

a. Study setting:

The recruitment for this study took place at the pediatric rheumatology clinic of Christian Medical College, Vellore. The OPD functions on 3 week days (Wednesdays, Fridays and Saturdays) a week. The OPD serves on an average about 40 patients which includes 5 -10 patients with systemic lupus erythematosus.

Children whose parents are willing to take part in the study are recruited after an informed written consent. Children were recruited over a period of 6 months from March 2012 to November 2012.

b. **Participants:**

Inclusion criteria:

*Children diagnosed to have systemic lupus erythematosus as per American College of Rheumatology criteria with proliferative lupus nephritis (Class III and IV) and treated with similar doses of prednisolone

*Children aged between 8 and 18 years

*Children who have received stable doses of MMF for atleast 1 month.

Exclusion Criteria:

*Children with SLE aged >18 years

*Children with SLE who had Class II or Class V Lupus nephritis

*Children taking MMF not on empty stomach or taking not on regular intervals

*Children who have developed significant side effects to MMF like diarrhoea and marrow suppression

*Children with lupus nephritis with concomitant drugs like cyclophosphamide, cyclosporine, tacrolimus or azathioprine

c. **Variables:**

Exposure: All children with proliferative lupus nephritis on MMF for at least one month (standard dose 50 mg/kg/day)

Outcome: MPA AUC correlates with disease activity as measured by SLEDAI

Predictors: Dose of MMF, Disease activity, C3, C4, Albumin, Creatinine

d. **Data Sources/measurement:**

Complete blood count: Sample is sent in an EDTA tube and sent to the clinical pathology lab. Blood is processed in the Beckwith coulter machine and the validated results are considered.

Serum creatinine: 2ml blood is collected in a clotted tube and sent to the biochemistry lab. Creatinine is calculated by the picric acid method. The values are measured in

Total complements/C3, C4: 2ml of blood is collected in a clotted tube and sent to microbiology lab. Serum complements (C3 and C4) are measured by the nephelometry method.

Ds-DNA: Blood is processed in the autoimmune lab by the ELISA method.

Urine protein creatinine ratio: Spot urine sample is processed in the biochemistry lab by the Pyrogall indicator method.

e. **Sample size:** A total of 25 children who fulfilled the inclusion criteria were recruited

f. **Statistical methods:**

- The quantitative variables were presented using the mean and standard deviation.
- All categorical variables were presented using the frequencies and percentages
- . The mean MPA values were compared across male and female using independent t test.
- The comparison of MPA across BSA values was compared using Mann Whitney U test. Spearman Rank correlation was used to find the relationship between MPA and all continuous variables.
- .All the variables that were significant from the bivariable analysis were included for multivariable regression analysis
- Data analysis were done with SPSS Version 17

Methodology:

Children with systemic lupus erythematosus with lupus nephritis who have been on MMF for the induction or maintenance therapy were inducted for the study. Most importantly children should have been on the drug for at least 1 month and the intake of drugs should be organized and appropriately spaced through the day. An informed, written consent was obtained for each patient after detailed one to one discussion with the parents/patient. After the consent, the child was examined.

After getting the informed written consent from the patient/ guardians the patient is asked to give blood for complete blood count, serum Creatinine, serum albumin, complements and anti-ds DNA.. The patient is also asked to give urine for routine analysis and for spot protein Creatinine ratio. After the results are available the disease activity is assessed with the SLE disease activity index scoring (SLEDAI) system. The patient is instructed to take the tablet at the usual dose and given instructions to come fasting and not to take the next day's morning dose. The patient is instructed to come to the clinical pharmacology unit the next day on an empty stomach. In the clinical pharmacology lab an IV cannula is inserted in situ and blood for trough MPA level is taken after which the line is flushed with heparin saline. The child is then instructed to take the drug. After the baseline (trough) sample, samples are taken at 0.5, 1, 1.5, 2, 2.5, 3, 4, 8 and 12 hours after MMF administration. The specimen will be centrifuged and plasma separated into a clean eppendorf tube. All specimens will be stored at $-20^{o}C$ until analysis.

Plasma MPA concentrations will be determined by high performance liquid chromatography (HPLC). Plasma concentration data will be used to estimate the concentration time curve (AUC_{0-12}). The patients' AUC 0-12 concentration is determined and compared with the clinical status and the serological status. It is postulated that a high drug concentration level will correspond with a lower disease status viz normal complements and a negative ds-DNA as well as clinical remission(86). It will also be tried to ascertain the optimal drug concentration to induce or maintain remission. To my knowledge there has not been any such study on the Indian population and very few studies overseas have been done on children with SLE.

This study is approved by Institutional Review Board (IRB) of Christian Medical College, Vellore (No 7702.)

RESULTS

RESULTS

Patient characteristics:

Twenty-five outpatients who fulfilled the inclusion criteria were recruited for the study. Out of 25 children, 20 were girls and 5 were boys (Fig1). Out of the twenty five, 16 children received 2gms/day, 7 received 1.5gms/day and 2 received 1gm/day. Indications for administering MMF were lupus nephritis class III (n=8), lupus nephritis class IV (n=17) (Fig2). On the day of study, 13 patients were receiving concomitant prednisolone and 22 children were receiving Hydroxychloroquine. SLEDAI scores done on all 25 children showed that 5 patients (21%) had active disease (SLEDAI score ≥6) and 20 patients (79%) had inactive disease (SLEDAI score<6) on the day of sampling (Fig3).

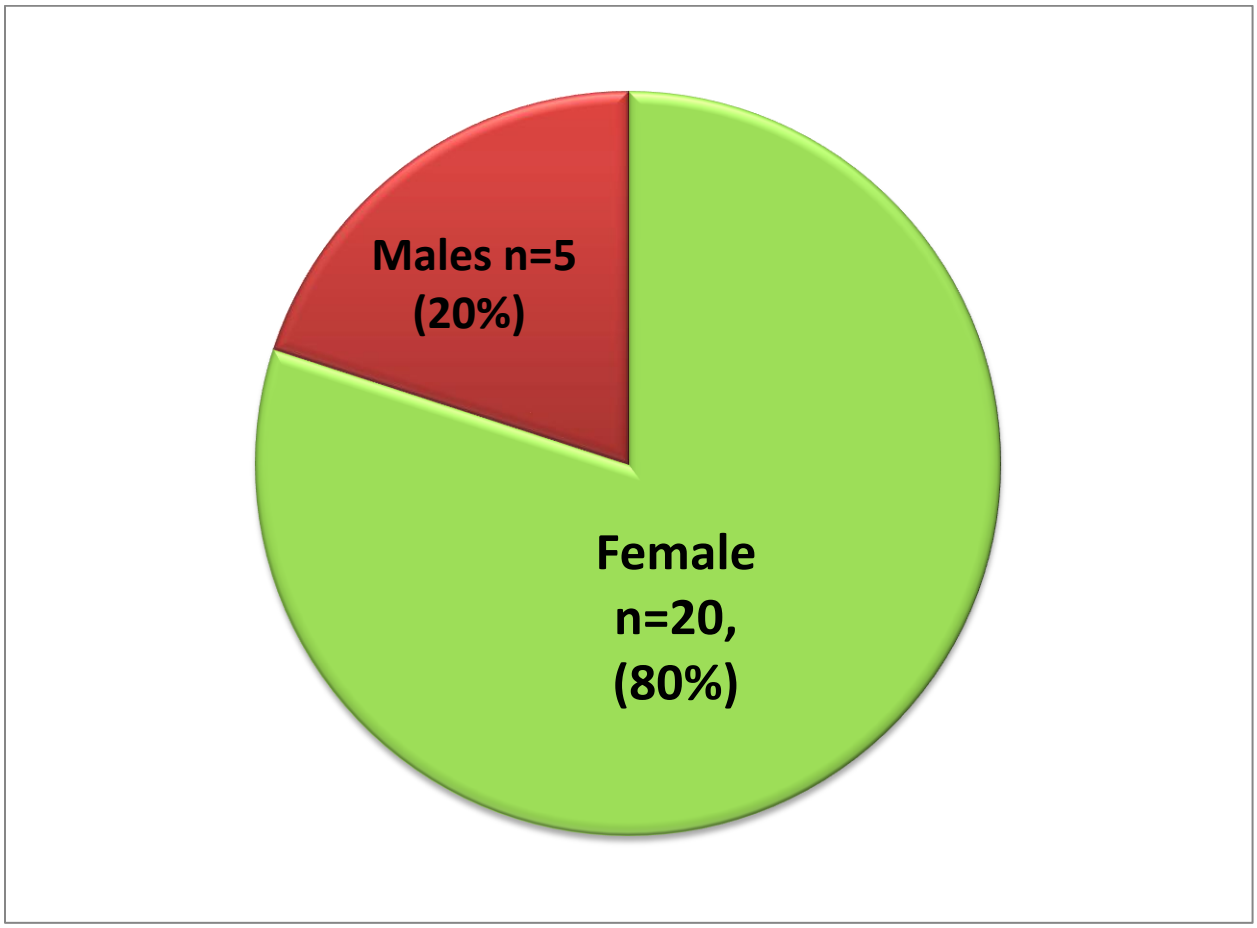
Table 1. Baseline characteristics of patients with active and inactive SLE

Parameters (Normal range)	**Active SLE (SLEDAI ≥6) n=5**	**Inactive SLE (SLEDAI <6) n=20**	**p value**
Age	15.0±3.3	14.75 ±1.7	0.272
Height (Cms)	151.8±12.3	152.9±8.8	0.974
Weight (Kgs)	48.6±12.39	48.35±12.21	0.767
Creatinine (0.7 to 1.1 mg/dl)	0.8±.18	0.78±.11	0.92
Serum Albumin (3.5 to 5 gm/dl)	4.08±.5	4.4±.36	0.272
Anti-ds-DNA (<100 IU/ml)	240±62	156.9±227	0.060
Total count /Cu.mm	8740±2967	8900±3078	1.000
Hemoglobin (gm%)	10.2±1.9	12.3±0.72	0.007
C3 level (90-180 mg/dl)	66.12±13.29	99.69±15.59	0.001
C4 level (10-40 mg/dl)	8.1±3.5	18.8±6.9	0.001

MPA AUC_{0-12}:= Mycophenolic acid Area under the curve 0-12 hours, Ds-DNA:=double stranded deoxyribonucleic acid

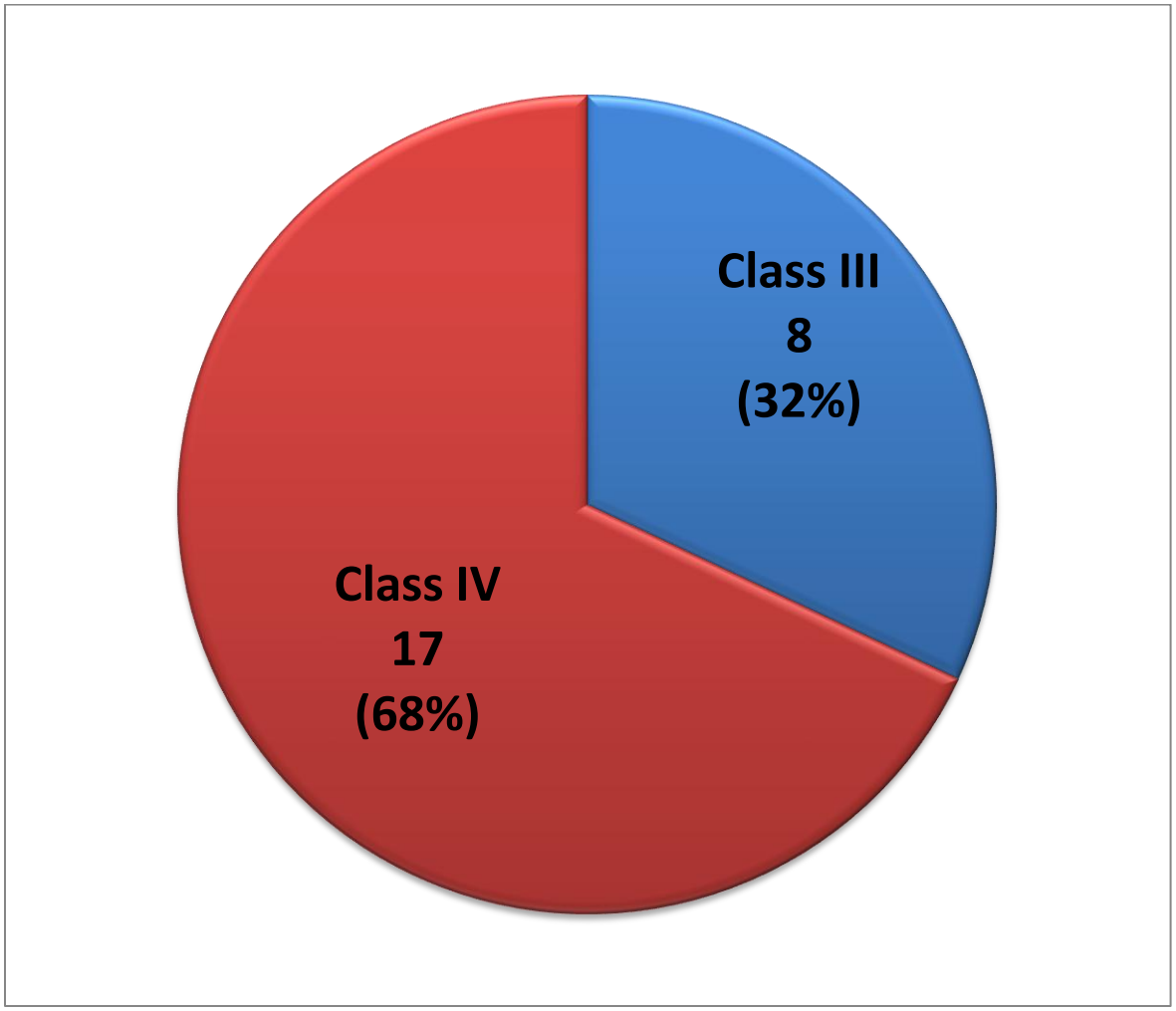
The active and inactive disease groups were similar in age (mean age was 15.0 ±3.3 in the active disease group and was 14.75±1.7 in the inactive disease group).The two groups were also similar in weight, height and serum albumin levels. Hemoglobin, C3 and C4 levels were statistically significant between the two groups ($p < 0.05$)

Figure1: Showing the sex distribution among the participants



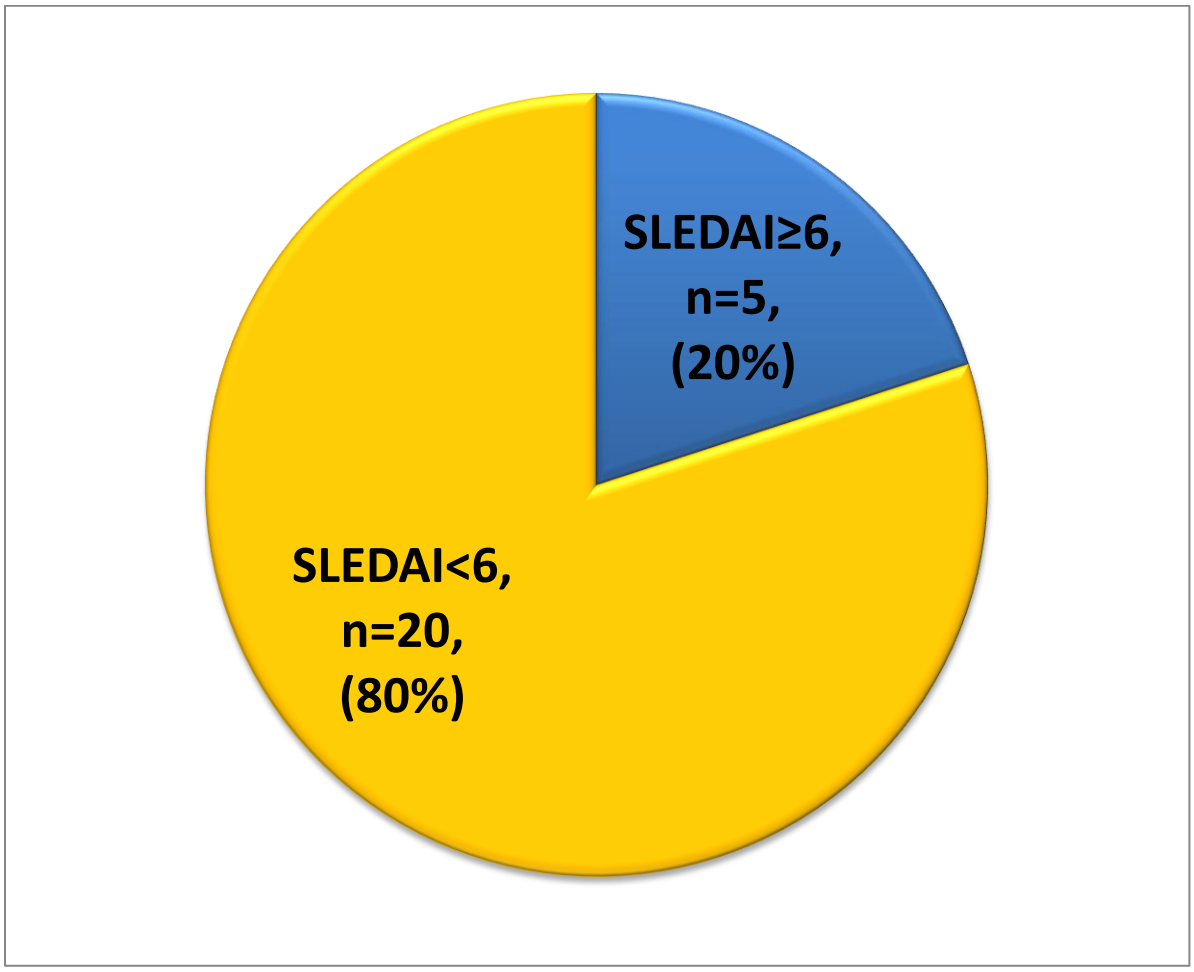
80% of our patients were girls (n=22) and 20% were boys (n=3)

Figure 2. Lupus nephritis class among the patients



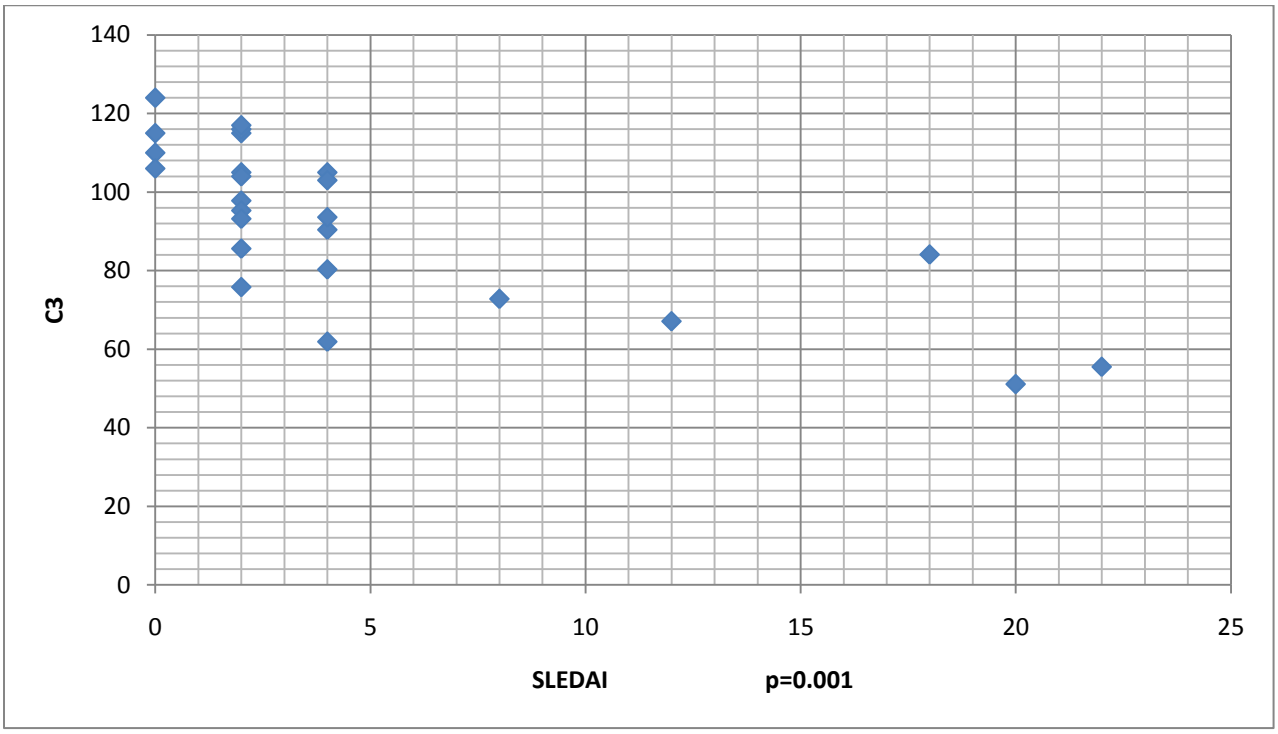
Our participants predominantly had class IV nephritis.

Fig3: Distribution of disease activity among the participants



80% of the participants (n=20) had inactive disease(SLEDAI score<6) on the day of examination.20% (n=5) had active disease (SLEDAI score≥6).

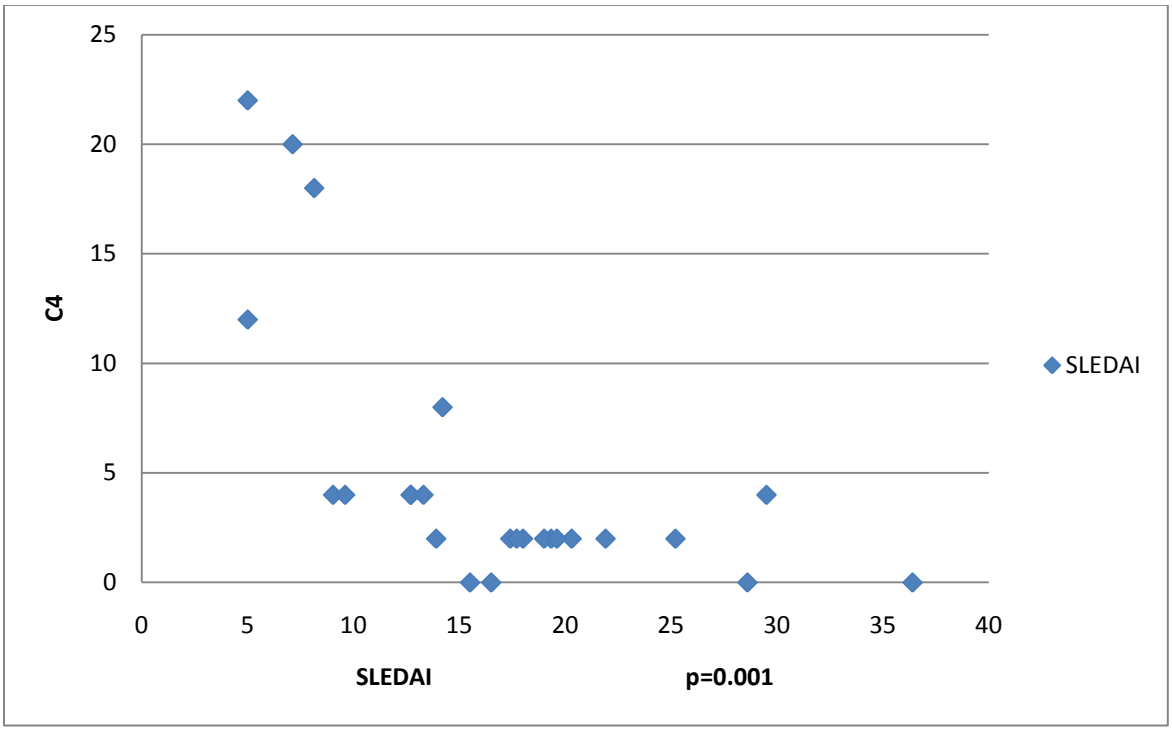
Figure 4. Correlation of C3 levels with SLEDAI



SLEDAI:=Systemic Lupus Erythematosus Disease Activity Index

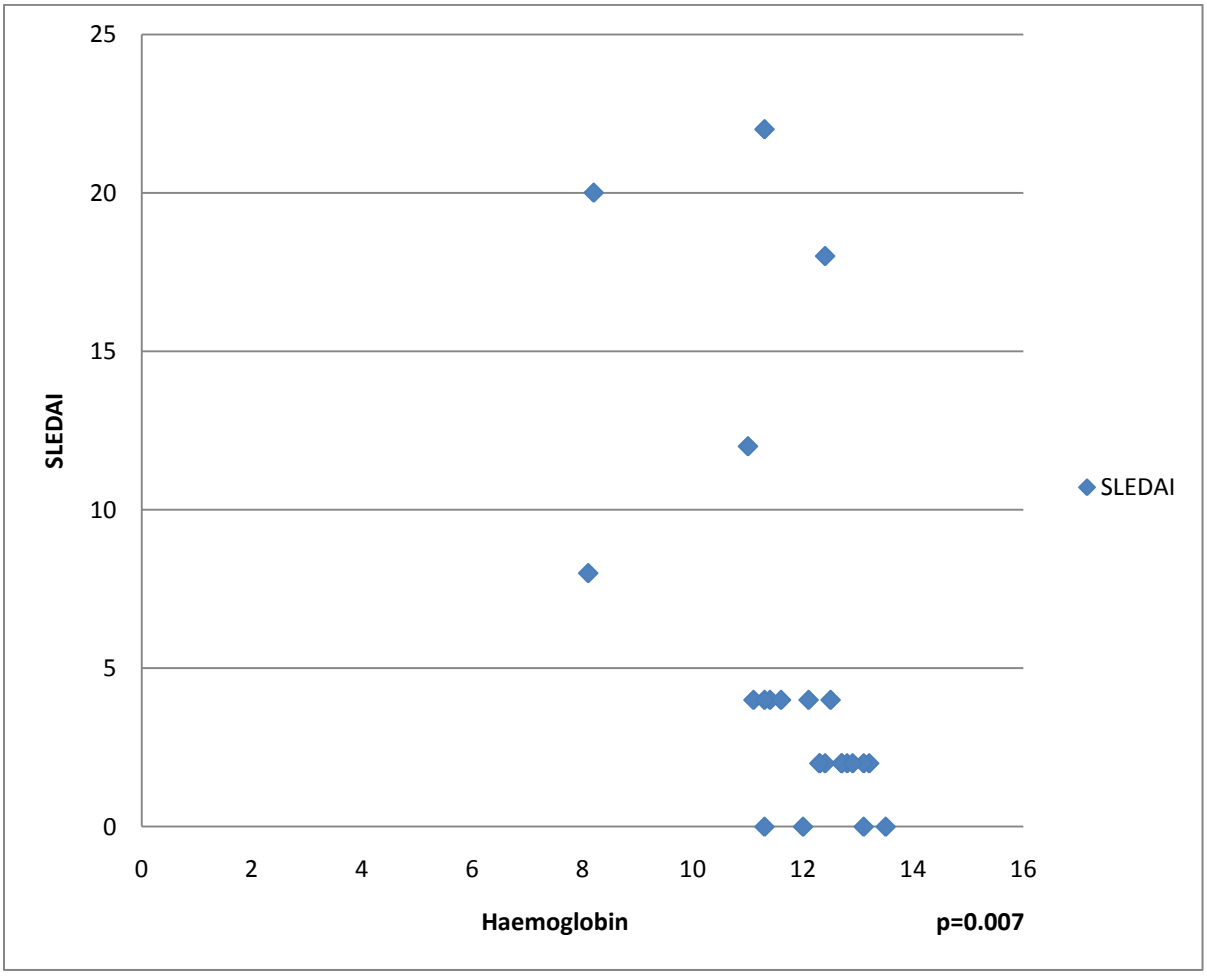
Low C3 was significantly correlated with high disease activity (P=0.001)

Figure 5. Correlation of C4 with SLE disease activity



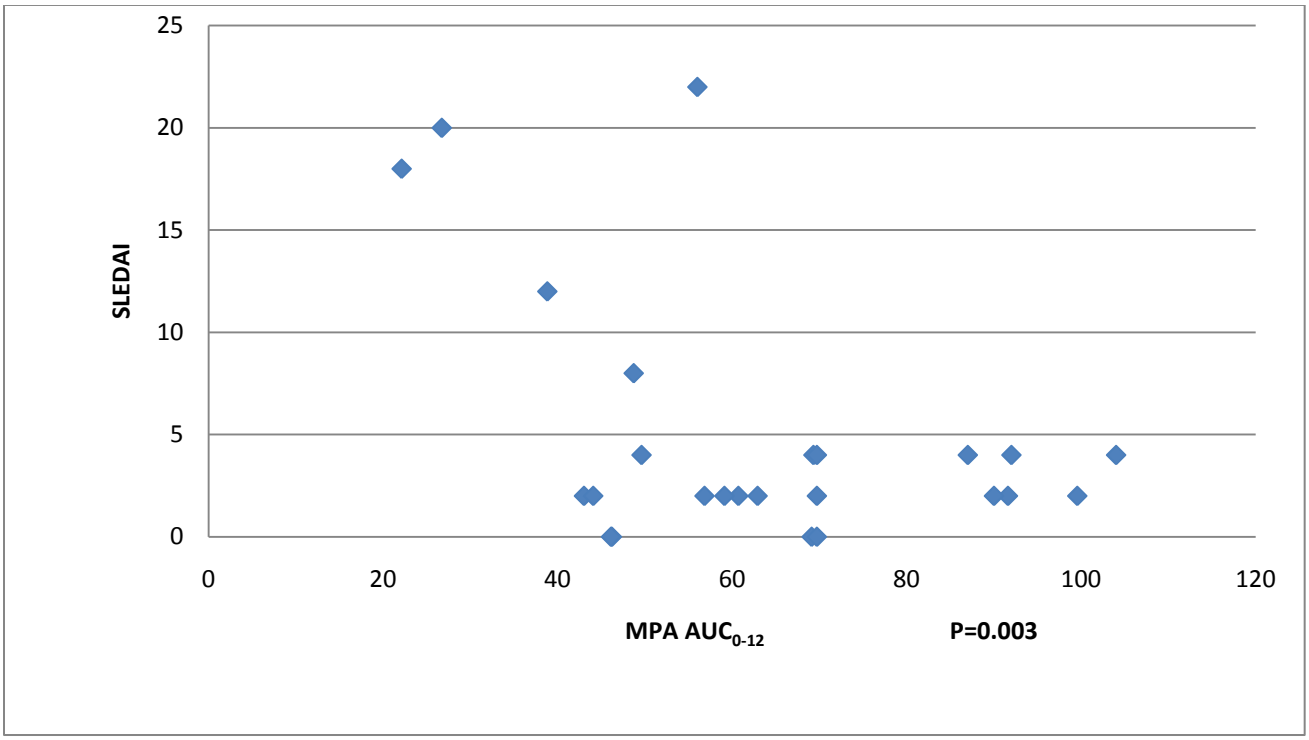
Low C4 was significantly correlated with high disease activity (P=0.001)

Figure 6: Correlation of hemoglobin and SLEDAI



Hemoglobin correlated significantly with disease activity (SLEDAI scores)

Fig7: **Correlation of MPA AUC_{0-12} with SLE disease activity**

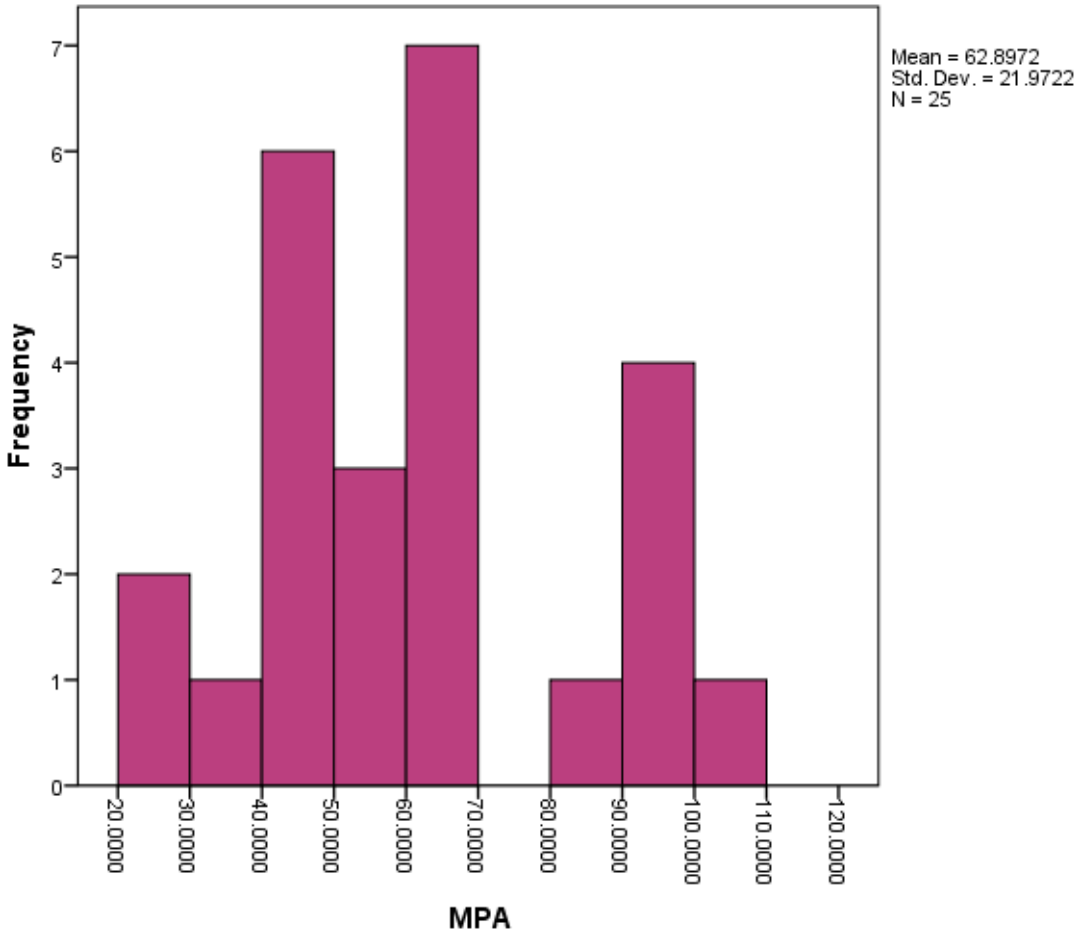


MPA AUC_{0-12}:= Mycophenolic acid Area under the curve 0-12 hours

High SLEDAI scores correlated with low MPA AUC_{0-12} on the day of examination(P=0.003)

Inter-Individual variability:

Fig8.Between-patient variability of MPA AUC_{0-12} /gm of MMF



MPA:-Mycophenolic acid

MPA AUC_{0-12} showed wide variability (Fig8) with lowest level of 22.1 and a highest level of 104µg.hr/ml. The mean ±SD MPA AUC_{0-12} of the active disease group (38.46±14.3µg.hour/ml) was significantly lower than that of the inactive group (69±19.24µg.hour/ml) with a p value of 0.003.

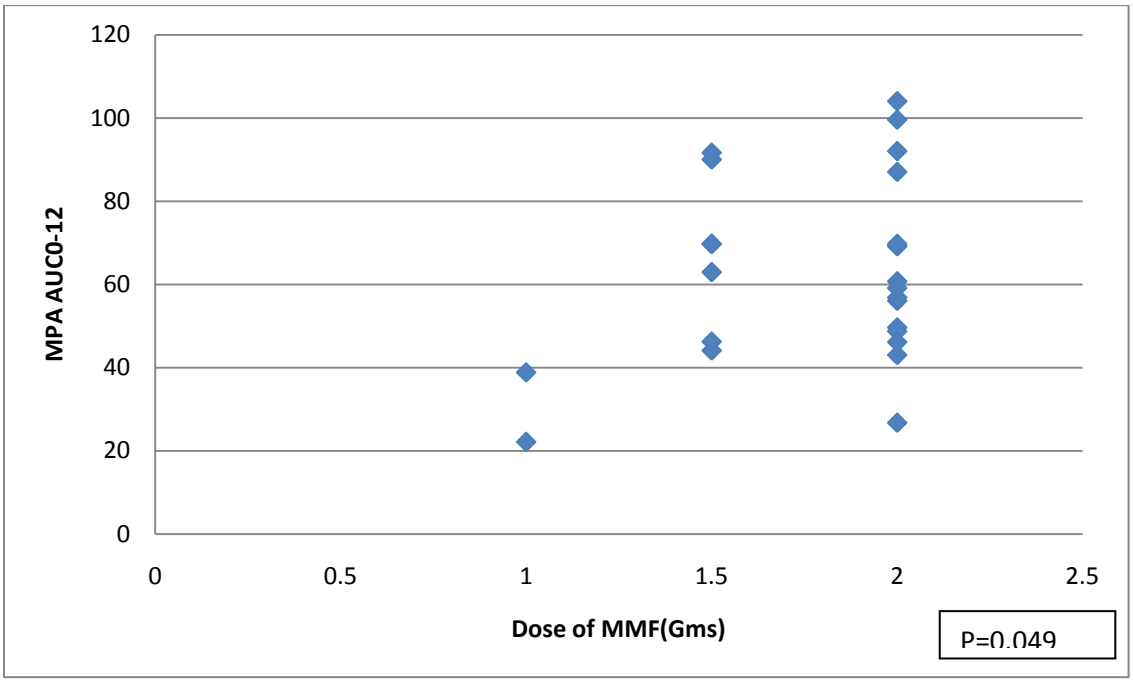
Table 2 Parameters influencing MPA AUC_{0-12} levels

		UNIVARIATE		MULTIVARIATE	
		SD	**P value**	**95%CI**	**P value**
Sex	**Male**	64.1±22.6	0.593	-28.9 - 18.2	.65
	Female	58.1±20.6			
BSA	**<1.5**	66±23.26	0.31	-33.8-52.3	.67
	>1.5	56.2±18.5			
Creatinine clearance	**<90**	56.0±0	0.8	-18.3 – 65.73	.27
	>90	63.1±22.4			

BSA: =Body Surface Area

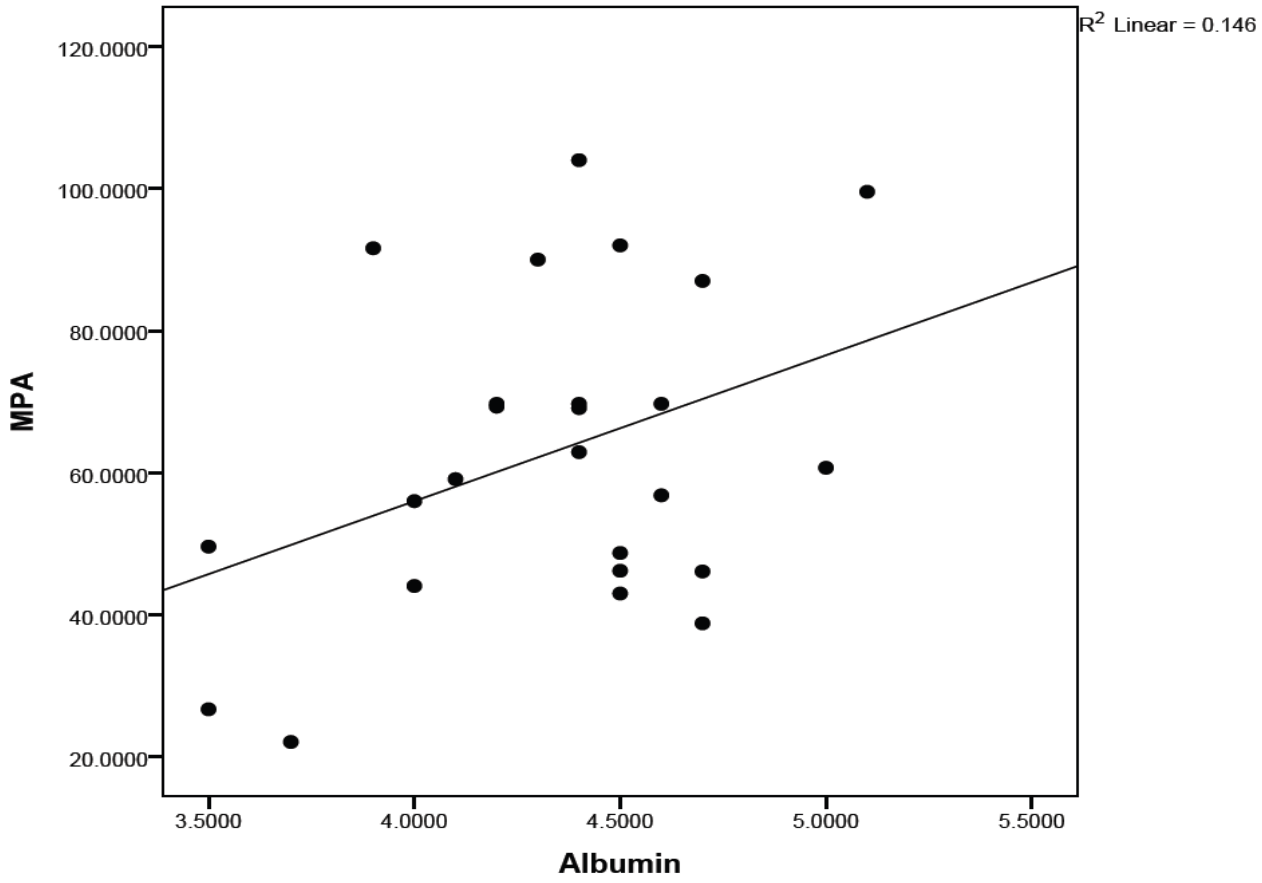
A univariate and multivariate regression was done to predict the factors influencing **MPA AUC_{0-12} levels**. Factors included were sex, body surface area, dose of MMF, albumin and C3 levels. Only MMF dose was found to be significantly influencing the MPA AUC_{0-12}.

Figure 9 Correlation of MPA AUC-$_{0\text{-}12}$ with dose of MMF



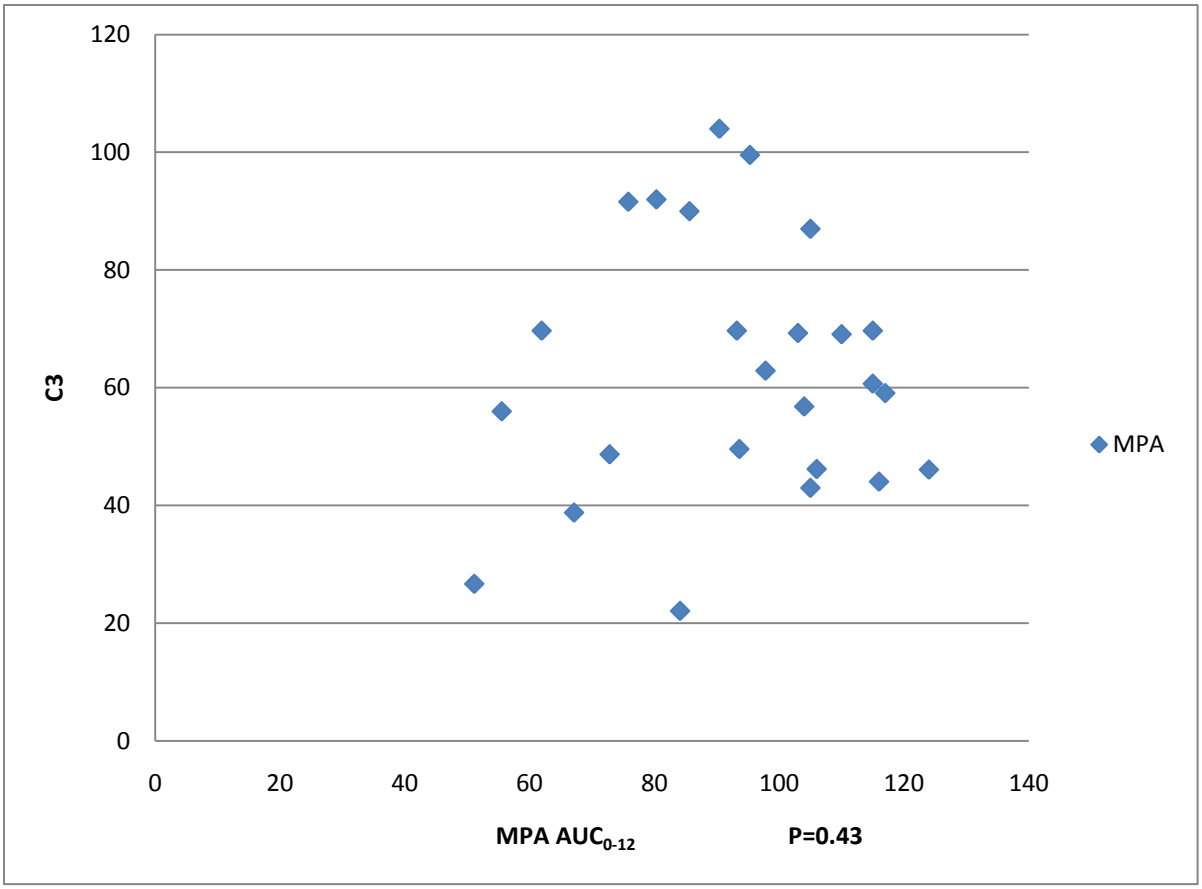
Higher the dose of MMF correlated to better MPA levels(Fig9).

Fig10. Correlation of albumin with MPA AUC $_{0\text{-}12}$



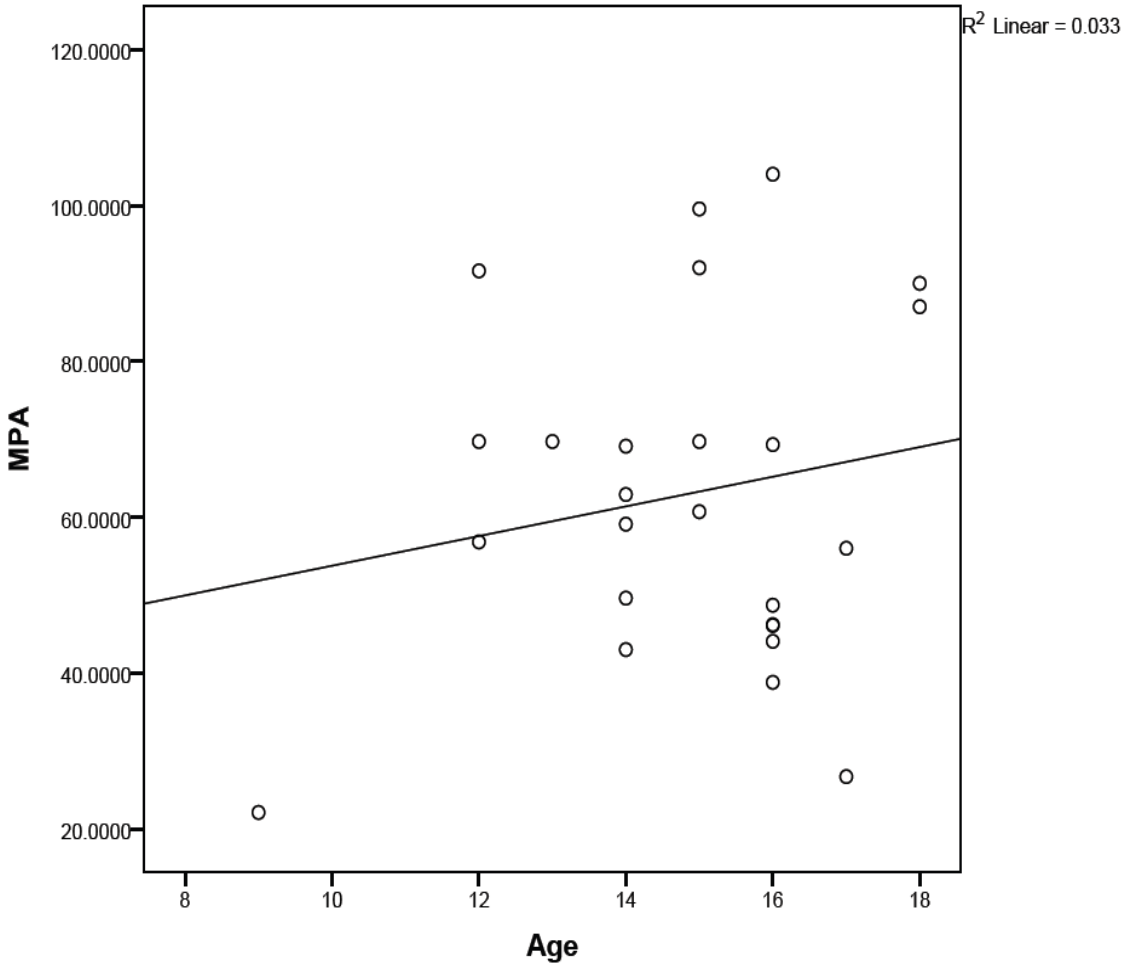
Serum albumin was not significantly associated with MPA $AUC_{0\text{-}12}$

Figure 11. Showing correlation of C3 with MPA AUC $_{0\text{-}12}$



C3 did not correlate significantly with MPA $AUC_{0\text{-}12}$ (fig 11) .

Figure 12. Showing correlation of age with MPA AUC $_{0\text{-}12}$



There was no significant correlation between age and MPA AUC $_{0\text{-}12}$

DISCUSSION

DISCUSSION

This is the first study to evaluate MMF pharmacokinetics in children with Lupus nephritis. There has been a dramatic improvement in the survival rates of patients with systemic lupus erythematosus. The 4 year survival rates in the 1950s was 50% and has currently the 15 year survival rate has improved to 80% with the novel treatment options(98). Among them is mycophenolate mofetil which has been routinely used in the solid organ transplant patients. MMF is as effective and has fewer side effects than steroids and cyclophosphamide(12).

However the ideal dose of MMF in children with lupus nephritis is not described. Traditionally a dose 2 to 3 grams per day in divided doses is given. A dose of 900mg/m^2 has been recommended in children as against a dosage of 1200 to 2400 mg/m2 in patients with solid organ transplants. These dosing are derived from that recommended for solid organ transplants and there are no randomized control trial derived doses available for the treatment of lupus nephritis(90). There are a number of literatures suggesting that weight or body surface area dosing of MMF is not advisable as it does not predict MPA pharmacokinetics and MPA Pharmacodynamics (99). In renal transplantation therapeutic drug monitoring has been developed for individualization of doses(90). The inter-individual variability of drug concentration of MMF is a characteristic feature of the drug. There are a number of factors that are described to affect the drug concentration like concomitant drug intake namely cyclosporine, tacrolimus and associated co morbidities like hypoalbuminemia and severe renal insufficiency(13).

In solid organ transplanted patients MPA area under the plasma concentration time curve from 0-12 hrs has been successfully correlated in the outcome (90). Notably the pharmacokinetics in SLE is different from the MPA pharmacokinetics of post solid organ transplants who are on Cyclosporin A (91,92) . There is a third peak in patients with SLE who have a third peak in the AUC due to the absence of calcineurin inhibitors(93,94). Individualized doses aiming at a Target concentration of AUC over 12h of 30-60 mg h/l when measured with high performance liquid chromatography or 35 to 70 mg h/l when measured by enzyme multiplied immunotechnique is advised for patient undergoing renal transplant (95). Target concentration of MPA that is aimed at for the therapy of lupus nephritis in adults is 30-60 mg h/L. The target concentrations of MPA in children treated with MMF for SLE is very important as this reflects the clinical outcome and the association with side effects. To our knowledge the target concentration of MPA in children with Lupus nephritis has not been discussed till date. Hence this study was done to demonstrate the inter-individual variability of MPA AUC_{0-12} levels in children taking MMF for lupus. There are no studies to our knowledge done in the pediatric age group in our country.

The setting of the study was at the pediatric rheumatology clinic which runs twice a week. It caters to an average of 40 patients per day among which systemic lupus erythematosus accounts for about 30% of the cases. The recruitment started in March 2012. Children with lupus nephritis who were on MMF for at least 1 month were included. Also the time spacing of the drugs were given importance. Children who had not been taking drugs equally spaced through the day were asked to come the next month after changing the drug timing. The reason for this is irregular therapeutic compliance accounts for variability of the drug levels.

25 patients who fulfilled the criteria were recruited over 8 months. 20 patients were girls which constituted 80% of the sample size and 5 were boys and contributed to 20% of the sample size. This was in concordance with the data about the global sex distribution of the disease although in children the female to male preponderance is not as significant as in adults. The female to male ratio is about 4.5:1 as against 8 -13:1 in adult onset patients(77). The peak onset of child hood SLE occurs during puberty(100). Our patient profile is in accordance with this finding. The mean age of our series of patient was 14.8 years.

The patients' height, weight and body surface areas were also recorded on the day of examination. Mycophenolate mofetil dose for treatment of lupus nephritis is derived from doses for solid organ transplants. Optimum doses for treating lupus nephritis patients are not well established. A dose of 2 to 3 grams per day in 2 divided doses is usually given. The pediatric transplant dose of 30mg/kg twice daily or 600mg/m^2 are being traditionally used(59,60). We used a dose of 600mg/m^2 for our patients. 64% of our patients had body surface area between 1-1.5 m^2. 32 % of our patients had a body surface area of more than 1.5 m^2 and 4% of the patients had body surface area less than 1 m^2.

All our patients who are on MMF have had lupus nephritis diagnosed and staged after a renal biopsy. 68% of the patients had class IV nephritis and 32% of them had class III nephritis. The indications for use of MMF among our patients were exclusively for lupus nephritis. Many studies report the successful use of MMF in the treatment of lupus nephritis(103). MMF is indicated in lupus nephritis, dermatitis and other manifestations of SLE(104,105).Among the studies the majority were on treatment of lupus nephritis with MMF after failed cycles of cyclophosphamide and has reported dramatic outcome. The corticosteroid sparing effect is of particular importance(9).

Concomitant medications that the child is on along with that of MMF were recorded. 56% of our patients were on prednisolone, 16% were on deflazocort, 84% were on hydroxychloroquine (HCQ) and 52% were on calcium and vitamin D supplements. None of them were on cyclosporine or tacrolimus. Plasma concentration of MPA is decreased by concomitant use of cyclosporine. Cyclosporine use inhibits the biliary excretion of MPA 7-0 – glucuronide which is the inactive MPA metabolite(106). MMF is often given in combination with other immunosuppressants and corticosteroids. Steroids contribute to the MPA metabolism and hence decreased the exposure over time(107).

On the day of examination the children are examined and the disease activity is assessed with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) . Recently, there are many disease activity indices that are validated(108). Among these are the British Isles Lupus assessment group (BILAG), the European Consensus Lupus activity measurement (ECLAM), the systemic lupus activity measure (SLAM) and the SLE disease activity index(SLEDAI) and the safety of estrogen in lupus erythematosus national assessment (SELENA) SLEDAI. Each of these indices are designed for longitudinal studies(109,110). In our study we decided to use the SLEDAI scoring system for the following reasons. The SLEDAI scoring system unlike BILAG is a global index and not organ specific and fewer number of variables (24 Vs 86)(111). Zahr et al in their study used both SLEDAI and BILAG index to assess disease activity and successfully correlated the MPA AUC_{0-12} with disease activity(86). In our study the patients were examined using the SLEDAI scoring system and then asked to come to the clinical pharmacology unit the next day.

In the clinical pharmacology unit, the child will have the mycophenolic acid assay. The child is then instructed to take the drug in the lab after the baseline (trough) sample. Later samples are taken at 0.5, 1, 1.5, 2, 2.5, 3, 4, 8 and 12 hours after MMF administration. The specimen will be centrifuged and plasma separated into a clean eppendorf tube. All specimens will be stored at –20^{o}C until analysis. We used the high performance liquid chromatography method (HPLC) for analysis of MPA AUC $_{0\text{-}12}$. HPLC is currently the preferred method for acute monitoring of the Mycophenolic acid assays(95). There are other methods to determine MPA levels namely the EMIT method. In a study done by Weber et al EMIT technique was found to be comparable to the HPLC method in determining the MPA levels. However there is cross reactivity with the metabolites of mycophenolic acid namely phenolic MPA glucuronide (MPAG) 7-o-MPAG, acyl glucuronide (AcMPAG) and phenolic glucoside of MPA(95).

Markers for active nephritis that we included were urine microscopy for blood and protein. For quantification of protein we used urine spot protein/Creatinine ratio done by pyrogall indicator method. We chose the spot protein Creatinine ratio as against the 24 hour collection mainly for the patient's convenience and the time it saves. Urine for spot protein Creatinine ratio is an alternative to the 24 hour urine protein method(112). In a study done in Asia, Absar et al concluded that single voided protein/creatinine ratio is an alternative to the 24 hour collection method at all levels of GFR(113). Any ratio > 2 was considered nephritic range of proteinuria. Proteinuria and hematuria are the most commonly associated findings in lupus nephritis(100). Hematuria is almost universal. Nephrotic range of proteinuria is found in 50% of patients with lupus at the time of presentation(5).

Creatinine was done for all patients prior to the MPA analysis. None of the 25 patients had renal insufficiency. Severe renal insufficiency is known to interfere with the MPA AUC $_{0\text{-}12}$(97). Creatinine was measured in the picric acid method and expressed in mg/dl. Albumin, complement levels were included to be independent variables that might influence the MPA AUC $_{0\text{-}12}$.

Complements were done for all the patients by the In the study done by Zahr et al multivariate analysis showed that Creatinine clearance dose of MMF and albumin was significantly associated with the MPA $AUC_{0\text{-}12}$. Complements (C3 and C4) were done by the nephelometry method. All co morbidities in the patient on the day of examination were looked for. None of our patients had any co morbidities.

In our study we have clearly shown a strong association between MPA AUC $_{0\text{-}12}$ and the disease activity as assessed by scoring the disease by the SLEDAI scoring system. In Zahr et al's study the MPA $AUC_{0\text{-}12}$ correlated with disease activity as assessed by both the SLEDAI and BILAG scoring(86). In the study by Rolland a similar finding was observed but however unlike the current study disease activity was not assessed by validated indices(114).

We found that the mean MPA $AUC_{0\text{-}12}$ was significantly lower in patients with active disease than the patients with inactive disease. It is notable that the two groups (active disease with SLEDAI>6 and inactive disease with SLEDAI <6) were similar in mean age, sex distribution, similar in concomitant medications intake.

We have demonstrated that MPA AUC $_{0\text{-}12}$ was correlated with SLEDAI, complement level (C3, C4) and hemoglobin. Complement level being one of the two main biological markers of SLE activity. Zahr et al found that complements, MPA AUC $_{0\text{-}12}$ along with ds-DNA were associated with SLE disease activity.(86)

In our multivariate analysis we found that daily MMF doses were recognized as independent variables influencing the MPA AUC $_{0\text{-}12.}$ It is very unlikely that other factors like duration of treatment with MMF or prior treatment with other drugs could have influenced the results since all the patients were on MMF for at least more than 8 weeks. The doses were not modified in the past 8 weeks. Moreover the frequencies of concomitant drug intake among the two groups were similar.

Therapeutic compliance is a critical issue. This may contribute to variability in the drug levels and disease activity(115). This was addressed at the time of recruitment itself. Only patients who were on regular medications and dosing were recruited.

Hence it can be said that the dosing of MMF needs to be based on the MPA AUC $_{0\text{-}12}$ levels. Body surface area and weight dosing might not attain adequate drug concentration and thereby not leading to good disease control.

We have also demonstrated the inter-individual variation of drug concentration per gram of MMF which substantiates that fixed daily doses are not recommended.

LIMITATIONS

Limitations of the study:

Since it is a cross sectional study the definitive conclusion regarding the association between MPA AUC_{0-12} levels and the SLE activity cannot be established. It can be done through regular therapeutic drug monitoring of MPA AUC_{0-12} levels.

CONCLUSIONS

Conclusion:

In conclusion, there is a strong correlation between the disease activity and the MPA AUC_{0-12} in children with lupus nephritis taking MMF. We have also demonstrated an inter-individual variability among patients taking a standard dose of MMF. In light of this the inclusion of MMF AUC_{0-12} as parameter in treating children with lupus nephritis patients with MMF is recommended. Also prospective pharmacokinetic study needs to be done to evaluate the target AUC_{0-12} for children with lupus nephritis taking MMF.

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